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Original Article

# Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties

Devanand L. Luthria[a,*], Marcial A. Pastor-Corrales[b]

[a]*Food Composition Laboratory, Beltsville Human Nutrition Research Center (East), Room 202 B, Building 161, BARC-E, 10300 Baltimore Avenue, ARS, USDA, Beltsville, MD 20705-3000, USA*

[b]*Vegetable Laboratory, 10300 Baltimore Avenue, ARS, USDA, Beltsville, MD 20705-2350, USA*

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**Abstract**

A high-performance liquid chromatography-diode array detection (DAD) procedure separating and quantifying 16 phenolic acids was used for determination of phenolic acids content in dry beans (*Phaseolus vulgaris* L.). Dry beans from 10 market classes and 15 varieties that are commonly consumed in the United States were screened for phenolic acids content. Systematic sequential hydrolysis of a model Black bean (Eclipse variety) showed an insignificant amount of free phenolic acids were extracted with a mixture of methanol and water (85:15,%v/v) containing 10% acetic acid. The majority of phenolic acids were extracted from the base hydrolyzed fraction, and further sequential acid hydrolysis of the same extract did not yield any additional amounts of phenolic acid. Therefore all bean samples were ground and hydrolyzed with base in the presence of ascorbic acid and ethylenediaminetetraacetic acid, and the free phenolic acids were extracted with ethyl acetate and analyzed by HPLC. Ferulic acid, *p*-coumaric acid and sinapic acid were detected and quantified in all varieties. However, caffeic acid was detected in measurable amount only in two Black bean varieties (T-39 and Eclipse). The average phenolic acid content of dry bean sample was determined to be 31.2 mg/100 g. Total phenolic acid content among all samples varied between 19.1 and 48.3 mg/100 g of bean samples. Ferulic acid was the most abundant phenolic acid present in all samples, whereas intermediate levels of *p*-coumaric acid and sinapic acid were extracted from all bean samples. Over 83% of the total phenolic acids were retained in bean samples during the cooking process, and only 2% or less were detected in water extracts during overnight soaking. Published by Elsevier Inc.

*Keywords:* Phenolic acids; Caffeic acid; *p*-Coumaric acid; Ferulic acid; Sinapic; HPLC; Ten beans class; Fifteen varieties; Soaking and cooking; Sequential hydrolysis; *Phaseolus vulgaris* L

## 1. Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes, consumed worldwide as pods of green beans or seeds of dry beans (Singh, 1999; Takeoka et al., 2003; Tasuda et al., 1994). The common beans are rich and inexpensive sources of proteins, carbohydrates, dietary fibers, minerals and vitamins to millions of peoples in developed and developing countries (Rehman et al., 2001). Dry beans are a staple food for many Latin American, Eastern and South African countries (Aggarwal et al., 2004). Total per capita consumption of dry beans has increased markedly over the past two decades (Lucier et al., 2000) in the United States. This increase is mainly due to a large immigration of Hispanic populations and to increased attention to beans being classified as functional foods. There are several literature reports linking bean consumption to reduced risk of cardiovascular disease, diabetes mellitus, obesity, cancer and diseases of digestive tract (Anderson et al., 1984; Geil and Anderson, 1994; Mejia et al., 1999; Bazzano et al., 2001). These potential health benefits of beans have been attributed to presence of secondary metabolites such as phenolic compounds that possess antioxidant properties (Cardador-Martinez et al., 2002; Lazze et al., 2003; Azevedo et al., 2003).

There are a number of reports in recent literature on extraction and analysis of polyphenols from fruits,

*Corresponding author. Tel.: +301 504 7247; fax: +301 504 8314.
*E-mail address:* luthriad@ba.ars.usda.gov (D.L. Luthria).

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vegetables, herbs and soybeans (Merken and Beecher, 2000; Cao et al., 1996). However, extraction, separation, structural elucidation and analyses of phenolic compounds from beans have received limited attention. Feenstra (1960) focused on anthocyanin pigments in the seed coat of dry kidney beans. Four anthocyanins, namely malvidin 3-gluocside, petunidin 3-glucoside, delphinidin 3-glucoside, and delphinidin 3,5-diglucoside were extracted from Black violet beans. Other researchers (Stanton and Francis, 1966; Okita et al., 1972) have reported isolation and characterization of anthocyanins pigments from different kidney bean varieties since then. Recent studies (Beninger and Hosfield, 1999, 2003; Cardador-Martinez et al., 2002; Choung et al., 2003; Romani et al., 2004; Heimler et al., 2005) have focused on antioxidant activity; germplasm characterization or separation; and structural elucidation of flavonol glycosides, isoflavones, and anthocyanins from bean samples and their contribution to the color of seed coat. In addition, Heimler et al. (2005) also compared the results between the two methods, rapid spectrophotometric methods for estimation of total phenolic content, total flavonoid content with HPLC (DAD) and HPLC/MS technique utilizing 12 bean samples collected from two different regions of Italy over a 3-year time period.

The challenges associated with the analysis of phenolic acids arise from their structural complexities, as these compounds may exist in multiple forms as free, esterified, glycosylated or polymerized (Robbins, 2003). In addition, these compounds are not uniformly distributed in plants at tissue, cellular and sub-cellular levels and may coexist as complexes with proteins, carbohydrates, lipids or other plants components. Hence, the polarity of phenolic acids varies significantly, and it is difficult to develop a uniform analysis procedure for an assay of all phenolic acids. The first reports on the determination of phenolic acids from bean samples were carried out primarily to evaluate the impact of phenolic acids on the organoleptic characteristics of four or fewer varieties (Drumm et al., 1990; Sosulski and Dabrowski, 1984; Schmidtlein and Herrmann, 1975). The authors used classical extraction procedures followed by base hydrolysis of bean extracts. No antioxidants were added during extraction and base hydrolysis to prevent the degradation of phenolic acids.

In continuing the research in our own laboratory on phenolic acids, we observed that Black beans contained significant amounts of phenolic acids. A systematic study was undertaken to separate, identify and quantify phenolic acids from 10 bean classes along with multiple varieties from three commonly consumed classes (Pinto, Great Northern and Black beans) in the United States. All extractions and analyses were carried out by recently developed procedures in the presence of ascorbic acid and ethylenediaminetetraacetic acid (Nardini et al., 2002; Robbins and Bean, 2004). The influence of soaking and cooking on the analysis of total phenolic acid content was also evaluated.

## 2. Materials and methods

### 2.1. Samples

Common dry bean from 10 different market classes, namely Pinto, Great Northern, Navy, Black, Dark Red Kidney, Light Red Kidney, Red Mexican, Cranberry, Pink and Alubia were provided by Dr. M.A. Pastor-Corrales of the vegetable Laboratory of the United States Department of Agriculture (Beltsville, Maryland). Three varieties of Pinto beans (Maverick, Buster and Othello), along with two varieties of Great Northern (Northstar, Matterhorn) and three Black-seeded varieties (T-39, Jaguar and Eclipse) were assayed for phenolic acid content.

### 2.2. Chemicals

Twelve phenolic acids and three aldehyde standards (gallic, vanillic, caffeic, ferulic, *p*-coumaric, *o*-coumaric, *m*-coumaric, protocatechuic, syringic, chlorogenic, *p*-hyroxybenzoic and sinapic acids and three aldehydes: vanillin, syringealdehyde, and protocatechualdehyde) were purchased from Sigma (St. Louis, MO, USA). Only 2,3,4-trihydroxybenzoic acid was purchased from Fluka Chemicals (Buch, Switzerland). HPLC-grade solvents, methanol and acetonitrile and analytical-grade ethyl acetate were purchased from Fisher Chemicals (Fair Lawn, NJ). HPLC-grade formic acid and sodium hydroxide were procured from Aldrich Chemical Company (Milwaukee, WI) and analytical grade hydrochloric acid was obtained from Fisher Chemicals (Fair Lawn, NJ). Deionized water (18 Ω) was prepared using a Millipore Milli-Q purification system (Millipore Corp., New Bedford, MA). Polyvinylidene difluoride (PVDF) syringe filters with pore size 0.45 μm were purchased from National Scientific Company (Duluth, GA).

### 2.3. Extraction of free phenolic acids and sequential hydrolysis of the same bean extract with a base followed by an acid

A single Black bean sample (Eclipse variety) was analyzed for free and conjugated phenolics acids. The conjugated phenolics acids were sequentially hydrolyzed by base followed by the acid as described by Mattila and Kumpulainen (2002). Ground dry bean sample (0.5 g) was treated with MeOH (7 mL) containing 0.2% TBH (2,3-*tert*-butyl-4-hydroxy anisole) and 10% acetic acid (85:15). The mixture was sonicated for 30 min and the volume of the extract was adjusted to 10 mL with distill water. Approximately, 1 mL aliquot of the bean extract was filtered through a 0.45 μm PVDF syringe filter and analyzed for free phenolic acid content by HPLC. The additional 9 mL of the bean extract was used for sequential hydrolysis experiment with base followed by an acid. Approximately, 10 mL of distill water and 5 mL of 10 M NaOH was added to the bean extract. The mixture was flushed with nitrogen

and stirred overnight at ambient temperature. The pH of the bean extract was adjusted to 2 by drop-wise addition of 6 N HCl, and the liberated phenolic acids were extracted with ethyl acetate (3 × 15 mL). The combined ethyl acetate layer was evaporated to dryness under nitrogen and the residue was re-dissolved in 1.5 mL methanol:water (75:25, % v/v) and analyzed by HPLC. The aqueous part from the base hydrolysis fraction was further treated with 25 mL of concentrated HCl and mixture stirred for 30 min in a hot water bath at 85 °C. The phenolic acids liberated after acid hydrolysis were extracted with ethyl acetate (3 × 15 mL). The combined ethyl acetate layer was evaporated to dryness and the residue was dissolved in 1.5 mL of methanol:water (75:25, % v/v) and analyzed by HPLC.

### 2.4. Base hydrolysis

All bean samples were ground in a coffee grinder and stored under nitrogen at −60 °C until analyzed. Approximately 200 mg of ground bean sample (particle size < 0.825 mm) was hydrolyzed by stirring ground sample with 5 mL of a basic solution (2 N NaOH) containing 10 mM EDTA and 1% ascorbic acid for 30 min at 40–45 °C (Nardini et al., 2002). The reaction mixture was acidified by adding 1.4 mL of 7.2 N HCl. The mixture was vortexed for 5–10 s and free phenolic acids were extracted with ethyl acetate (2 × 6.4 mL). The combined organic layer was evaporated to dryness under a steady stream of nitrogen. The residue was re-dissolved in 2 mL 75:25 methanol:water (% v/v). The vial was vortexed for 30 sec (three times). The extract was filtered through a PVDF syringe filters (0.45 μm) and analyzed by HPLC.

### 2.5. Soaking and cooking of two bean samples

A quantity of 20 g of black beans (T-39 variety) and Great Northern (Matterhorn variety) were soaked separately overnight in 50 mL of deionized water. After overnight soaking, the soaking water was collected and analyzed for phenolic acid content. The soaked beans were simmered for 1 h over medium heat in approximately 100 mL water. The cooked beans were meshed into paste and analyzed by HPLC. Both bean paste (200 mg) and bean-soaked water extract (2.5 mL) were hydrolyzed with base in presence of EDTA and ascorbic acid. Free phenolic acids were extracted with ethyl acetate as described above. The ethyl acetate extract fraction was analyzed by HPLC. Triplicate analyses were carried out with each bean sample (paste and bean-soaked water extract).

### 2.6. Separation and analysis of phenolic acids by HPLC

A high-performance liquid chromatography-diode array detection (DAD) procedure separating and quantifying 13 phenolic acids and three aldehydes, which was developed in our laboratory, was used for the assay of phenolic acids content in dry beans (Robbins and Bean, 2004). Bean extracts were analyzed on an HPLC system (Beckman Coulter, System Gold) coupled to a programmable detector (System Gold, series 166) and an autosampler (System Gold, series 508) operated by a 32 Karat software package. A reversed phase $C_{18}$ Luna column (Phenomenex, 150 × 4.6 mm; particle size 5 μm), preceded by a guard column (Phenomenex, 4 × 3.0 mm) of the same stationary phase was used for HPLC analysis. The column and the guard column were thermostatically controlled at 25 °C and the flow rate was set to 0.7 mL/min. The mobile phase consisted of two solvents; 0.1% formic acid (A) and methanol (B). The solvent gradient in volumetric ratios was as follows: 5–30% B over 50 min. The solvent gradient was held at 30% B for additional 15 min and at 65 min gradient was increased to 100% B. It was maintained at 100% B for an additional 10 min to clean up the column. Dual wavelengths (270 and 325 nm) were used to detect the eluent composition. HPLC analysis at 325 nm was used for quantification of the peak areas of four identified phenolic acids namely, *p*-coumaric acid, ferulic acid, sinapic acid, and Caffeic acid.

### 2.7. Calibration curves

All identified phenolic acids were quantified with external standards by using HPLC analysis as described previously. Six different standard stock solutions with varying phenolic acid concentrations were prepared for all four of the identified phenolic acids (caffeic, ferulic, *p*-coumaric and sinapic) isolated and extracted from the bean samples. The concentration of phenolic acids extracted from bean samples was calculated using the developed calibration curves equations as reported in the earlier publications from our laboratory (Robbins and Bean, 2004).

## 3. Results and discussion

Fig. 1 shows the HPLC chromatogram of the 13 phenolic acids and three aldehydes (gallic, vanillic, caffeic, ferulic, *p*-coumaric, *o*-coumaric, *m*-coumaric, protocatechuic, syringic, chlorogenic, *p*-hyroxybenzoic, 2,3,4-trihydroxybenzoic and sinapic acids and three aldehydes: vanillin, syringealdehyde, and protocatechualdehyde) standard mixture monitored at 270 nm and 325 nm. The structures of the 13 phenolics acids and three aldehydes were confirmed by comparison of their UV spectra and retention time with the authentic standards as described in the earlier publication from our laboratory (Robbins and Bean, 2004).

In order to determine the phenolic acid content of beans, a single Black bean sample (Eclipse variety) was systematically hydrolyzed by a sequential procedure to separate and identify free and conjugated phenolic acids by the procedure described by Mattila and Kumpulainen (2002). Fig. 2 shows the HPLC profiles from three extracts (free, base and acid hydrolyzed) for a single Black bean sample.

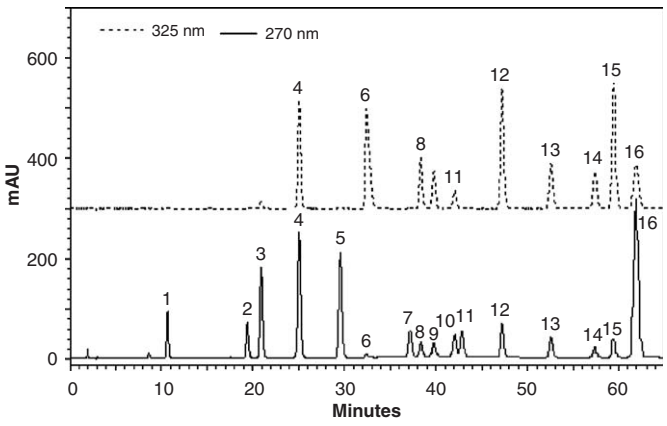


Fig. 1. A high-performance liquid chromatography-diode array detection (DAD) procedure for separating 13 phenolic acids and three aldehydes standard mixture monitored at two different wavelength (270 and 325 nm). All peaks were identified by comparison of retention time and UV spectra with commercial standards as follows: 1. Gallic acid, 2. Protocatechuic acid, 3. 2,3,4,-Trihydroxybenzoic acid, 4. Protocatechuic aldehyde, 5. *p*-Hydroxybenzoic acid, 6. Gentisic acid, 7. Vanillic acid, 8. Chlorogenic acid, 9. Caffeic acid, 10. Vanillin, 11. Syringic acid, 12. Syringealdehyde, 13. *p*-Coumaric acid, 14. Ferulic acid, 15. Sinapic acid, and 16. *m*-Coumaric acid.

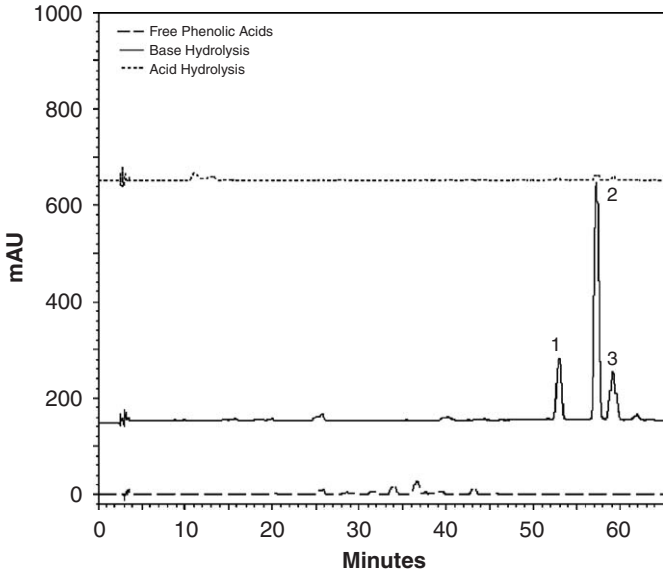


Fig. 2. High-performance liquid chromatogram profile of phenolic acid extracted from sequential hydrolysis experiment of a Black bean sample (Eclipse variety). Peaks 1, 2 and 3 were identified as *p*-coumaric acid, ferulic acid, and sinapic acid, respectively.

The HPLC eluent was monitored at two different wavelengths (270 and 325 nm). The HPLC results showed that insignificant amounts of free phenolic acids were extracted in the free phenolic acid fraction. However, base hydrolysis of the bean extract provided the majority of the phenolic acids, and the sequential acid hydrolysis did not yield any significant additional amount of free phenolic acids.

Based on the results obtained from the sequential hydrolysis experiment with Eclipse variety of the Black bean, only base hydrolysis was carried out with all the other varieties. The previous reports on determination of phenolic acids from bean samples also utilized base hydrolysis; however, no ascorbic acid and EDTA were added. Recently, Nardini et al. (2002) showed improvement in phenolic acids yields for base hydrolysis in the presence of ascorbic acid and EDTA. In the current study, base hydrolysis for all samples was performed in the presence of ascorbic acid and EDTA by the similar procedure as described by Nardini et al. (2002). However, the extraction of free phenolic acid with ethyl acetate was performed by vortexing the extract ($2 \times 1$ min), instead of sonicating the mixture with ethyl acetate (20 min). This modification reduced the total extraction time by over 30 min without any effects on the yield of phenolic acids.

The results of the phenolic acid content of the 15 bean samples are presented in Table 1. The three phenolic acids that were present in all bean classes and varieties; were identified as *p*-coumaric acid, ferulic acid and sinapic acid. Caffeic acid was identified in quantifiable amount only in two varieties, namely T-39 and Eclipse of Black bean class. Ferulic acid was the most predominant phenolic acid, and intermediate levels of *p*-coumaric acid and sinapic acid were detected in all bean varieties. Only *p*-coumaric acid and ferulic acid were reported in the initial report by Sosulski and Dabrowski (1984) from the flour and hull of the Navy bean. The total amount of phenolic acids extracted was 6.9 mg/100 g of dry bean samples (Sosulski and Dabrrowski, 1984). In a later study, the same authors reported extraction and identification of four phenolic acids (ferulic, *p*-coumaric sinapic and cinnamic acids) in significant higher amounts (19.1 mg/100 g) in dried canned Navy beans (Sosulski and Dabrrowski, 1990). In the current study, three phenolic acids were observed, namely, ferulic acid, *p*-coumaric acid and sinapic acid in all varieties of the beans analyzed. The total quantity of phenolic acids isolated in the current study from Navy beans (Vista variety) was 48.3 mg/100 g as compared to 19.1 mg/100 g reported in the earlier study (Sosulski and Dabrowski, 1984). This difference in total phenolic acids amount can be attributed to various factors such as variety, assay procedure, and growing and storage conditions (Dixon and Paiva, 1995; Ninfali and Bacchiocca, 2003; Hakkinen and Torronen, 2000). It is well documented that the quantity of phenolic compounds in foods is influenced by genotype (cultivar or variety), agronomic practices (irrigation, fertilization, pest management), maturity at harvest, post-harvest storage and climatic conditions (Dixon and Paiva, 1995; Ninfali and Bacchiocca, 2003; Hakkinen and Torronen, 2000). The average total phenolic acid content for all bean samples was determined to be 31.2 mg/100 g. The total phenolic acid content among all 15 samples varied between 19.1 and 48.3 mg/100 g of bean samples. The highest amounts of total phenolic acids were identified in Vista bean and two varieties of Black beans (T-39 and

Table 1
Determination of phenolic acid content in 15 varieties of commonly consumed dry bean in United States by HPLC analysis with diode array detection

| Bean class | Cultivars | Phenolic acid concentration (mg/100 g) ($n = 3$) | | | | | | | | Total phenolic acid content (mg/100 g) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Caffeic acid | | *p*-coumaric acid | | Ferulic acid | | Sinapic acid | | |
| | | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. | |
| Pinto | Maverick | | | 4.5 | 0.3 | 22.9 | 2.1 | 8.5 | 0.4 | 36 |
| | Buster | | | 4.5 | 0.1 | 16 | 0.3 | 9 | 0.3 | 29.5 |
| | Othello | | | 5.6 | 0.3 | 15.2 | 1.2 | 5.9 | 0.2 | 26.7 |
| Great Northern | Norstar | | | 4 | 0.4 | 17 | 2.3 | 9.4 | 2.1 | 30.4 |
| | Matterhorn | | | 6.3 | 0.9 | 17.2 | 0.6 | 9 | 1.4 | 32.5 |
| Navy | Vista | | | 12.4 | 2.5 | 26.6 | 2.3 | 9.2 | 1.7 | 48.3 |
| Black | T-39 | 1.1 | 0.1 | 11.6 | 0.6 | 25.5 | 1.6 | 9 | 0.7 | 47.1 |
| | Jaguar | | | 7 | 1.4 | 11.7 | 2.5 | 5.7 | 0.7 | 24.4 |
| | Eclipse | 1.1 | 0.1 | 9.8 | 0.4 | 24.7 | 0.3 | 6.8 | 0.2 | 42.5 |
| Dark Red Kidney | Red Hawk | | | 1.8 | 0.1 | 15.3 | 1.1 | 3.8 | 0.3 | 20.9 |
| Light Red Kidney | Cal Early | | | 7 | 0.2 | 14.8 | 0.5 | 5.7 | 0.2 | 27.4 |
| Red Mex | UI 239 | | | 5.8 | 0.1 | 17.4 | 0.6 | 5.4 | 0.2 | 28.6 |
| Cranberry | Taylor Cranberry | | | 1.7 | 0.1 | 14 | 1.1 | 3.5 | 0.3 | 19.1 |
| Pink | UI 537 | | | 6.8 | 0.7 | 19.4 | 1.7 | 8.2 | 0.5 | 34.4 |
| Alubia | Beluga | | | 5.3 | 0.1 | 10.6 | 0.7 | 4 | 0.3 | 19.8 |

Eclipse). However, total phenolic acid content in Jaguar variety was approximately 50% as compared to the two other varieties (T-39 and Eclipse) of the same Black bean. The lowest amount of the total phenolic acid content was determined in the Cranberry bean class (Taylor Cranberry variety). Intermediate levels of phenolic acids were determined in all other varieties. Ferulic acid was the predominant phenolic acid in all bean varieties assayed in the current study. The mean ferulic acid amount in all bean varieties was determined as 17.8 mg/100 g of dry bean samples as compared to 6.3 and 7.0 mg/100 g of *p*-coumaric acid and sinapic acid, respectively. Caffeic acid was detected in quantifiable amounts (1.1 mg/100 g) only in two Black bean varieties (T-39 and Vista).

Black bean (T-39 variety) and Great Northern (Matterhorn variety) were selected as two model bean substrates for soaking and cooking experiment. The results of the soaking and cooking experiments are described in Table 2. Only three phenolic acids (ferulic acid, *p*-coumaric acid and sinapic acid) were identified in the soaked water and cooked bean paste samples for both bean varieties. Over 83% of the total phenolic acids were retained in the cooked bean, and only minor amounts (<2%) were identified in the soaked water fraction for Black bean sample. Soaking process aids in softening the dried beans by returning the moisture to the dried beans. Soaking also reduces the cooking time and makes beans easier to digest. As the total amount of phenolic acid content in soaked water was less than 2%, no significant amount of nutritional value attributed by phenolic acids was lost during overnight soaking of dried beans. Only 85% of the total phenolic acids were accounted for in the two extracts (water and bean paste) for Black bean. The additional 15% of total phenolic acid may either be oxidized during the cooking process or else converted to other phenolic compounds that were not assayed by the current HPLC procedures. The results with the Great Northern (Matterhorn variety) cooking experiment were quite similar to Black bean samples, as over 99% of the phenolic acids were retained in

Table 2
Determination of phenolic acid content in soaked water extract and cooked bean paste of two bean samples

| Sample | *p*-coumaric acid (mg/100 g) | Ferulic acid (mg/100 g) | Sinapic acid (mg/100 g) | Total phenolic acids (mg/100 g) |
|---|---|---|---|---|
| Black bean (T-39 variety) | | | | |
| *Soaked water* | | | | |
| Mean ($n = 3$) | 0.3 | 0.5 | 0.1 | 0.9 |
| S.D. | 0 | 0 | 0 | 0.1 |
| *Paste of simmered beans* | | | | |
| Mean ($n = 3$) | 10 | 20.1 | 8.1 | 38.2 |
| S.D. | 0.7 | 0.9 | 0.5 | 2.2 |
| Great Northern (Matterhorn variety) | | | | |
| *Soaked water* | | | | |
| Mean ($n = 3$) | 0.1 | 0.1 | 0.0 | 0.2 |
| S.D. | 0.0 | 0.0 | 0.0 | 0.0 |
| *Paste of simmered beans* | | | | |
| Mean ($n = 3$) | 5.1 | 16.3 | 10.8 | 32.2 |
| S.D. | 0.4 | 1.3 | 1.0 | 2.7 |

1. Black bean (T-39 variety) and 2. Great Northern (Matterhorn variety).

the cooked bean paste and less than 1% of total phenolic acids were recovered from the soaked water extract. However, the quantitative recovery of total phenolic acid was obtained from the Great Northern (Matterhorn variety) cooking experiment.

## 4. Conclusions

The phenolic acid profile and total phenolic acid content in 10 classes and 15 varieties of beans (*Phaseolus vulgaris* L.) commonly consumed in United States of America were determined by high-performance liquid chromatography-diode array detection (DAD) procedure. Ferulic acid, *p*-coumaric acid and sinapic acid were the three main phenolic acids identified in all classes and varieties; quantifiable amounts of caffeic acid was identified only in two varieties (T-39 and Eclipse) of the black bean class. Variable quantities of total phenolics acids were determined in different classes. The maximum amount of total phenolic acids was identified in Vista bean and two varieties of black bean (T-39 and Eclipse); the minimum amount was identified in Cranberry bean class (Taylor Cranberry variety).

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